STUDIES ON IMMUNITY IN TYPHUS EXANTHEMATICUS WITH REFERENCE TO THE ANTIBODIES IN MAN AND GUINEA-PIG DEMONSTRABLE BY THE DALE METHOD *

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In the studies by Plotz, Olitsky, and Baehr[1] on the etiology of typhus fever, one of us reported the occurrence of agglutinins, complement-fixing bodies, precipitins, and opsonins in the blood of typhus-fever patients. It is significant that all types of antibodies occurred regularly after the crisis, occasionally at the crisis, and only rarely before the crisis. These antibodies usually persisted in the blood many months after convalescence. In the blood of the guinea-pig, to which the disease is transmitted by the injection of human virus, no antibodies except opsonins could be demonstrated.

The present paper is concerned with the study of the serum of patients and of guinea-pigs suffering or recovering from typhus fever, by a method involving the anaphylactic response. This method was first used by Dale[2] in the study of anaphylactic phenomena. It depends on the muscular contraction of the uterus of a sensitized guinea-pig when brought in contact with the antigen. Briefly, the technic of all such experiments is as follows: The uterus is removed from the animal and suspended in a container filled with Locke's solution, kept at a temperature of 37-40 C.; it is attached to a lever which writes on a moving drum. The antigen is added to the Locke's fluid bathing the uterus, and a contraction is recorded by the up-stroke of the lever. Ergamine is used to test the contractility of the muscle. This method was first successfully applied to the study of infectious diseases (pneumonia) by Weil.[3]

Antigens prepared by various methods were tried. The following proved the most satisfactory. The 7-day anaerobic growth on 0.5% glucose serum agar of Bacillus typhi-exanthematici was emulsified in physiologic salt solution,

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[1] Jour. Infect. Dis., 1915, 17, p. 1.
[2] Jour. Pharmacol. and Exper. Therap., 1913, 4, p. 167.
[3] Weil and Torrey, Jour. Exper. Med., 1916, 23, p. 1.

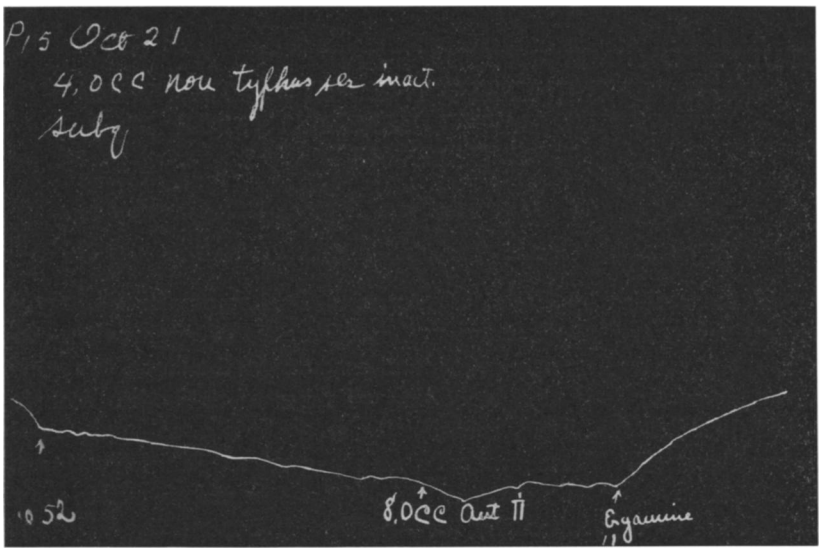

Fig. 1. The titration of the antigen. Guinea-pig 15 received 4 c.c. of inactivated non-typhus serum subcutaneously on October 18. On October 21, the uterus was tested; no response to 8 c.c. of antigen. Approximate dose of antigen 4 c.c.

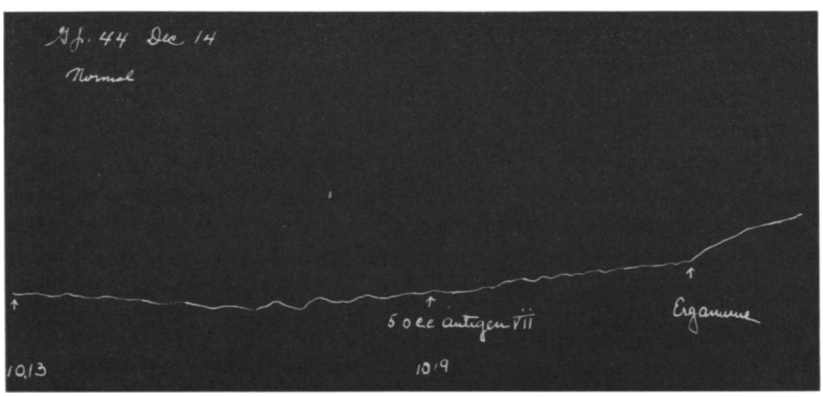

Fig. 2. The titration of the antigen. Guinea-pig 44 normal. No reaction to 5 c.c. of antigen.

washed 6 times in 0.9% salt solution, and finally suspended in distilled water. This suspension was incubated for 24 hours at 37 C., sealed in sterile tubes, and kept on ice. Many substances, human, horse, and rabbit sera, when applied in sufficient dosage to the guinea-pig uterus suspended in a Dale apparatus, cause a contraction by virtue of their irritant action. Each antigen was therefore tested against the uterus of a normal guinea-pig to determine the largest dose that would fail to induce a contraction. One-half this amount or less was used in the experiments on injected animals (Figs. 1 and 2).

The antigens were also tested against the uteri of guinea-pigs injected with normal serum. It will be recalled that Bacillus typhi-exanthematici is grown on human-serum (ascitic fluid) media; even the most thorough washing may fail to eliminate traces of serum clinging to the bacterial bodies used as antigen. Reactions between traces of serum in the typhus antigen and the antibodies developed in the guinea-pig against the injected human serum must therefore be excluded. Antigens which gave a positive response were discarded and only those used which failed to cause a contraction in the uteri of guinea-pigs injected with normal serum. In the end the complications arising from the possible presence of human serum in the antigen were altogether eliminated by the production of typhus fever in guinea-pigs and by the testing of such animals with antigens prepared as described.

The antigen was also tested for its antigenic properties—its capacity for reacting to specific antibodies. This was demonstrated in the following way: Rabbits were immunized against the typhus bacillus by the repeated injection of living organisms and 10 days after the last injection, the animals were bled. Two cubic centimeters of this immune serum were injected subcutaneously into female guinea-pigs and after an interval of 3 days, the uteri were tested for response to the antigen. The contraction was in each case striking, indicating the capacity of the antigen to react to antibodies developed for Bacillus typhi-exanthematici.

ANTIBODIES IN PATIENTS WITH TYPHUS FEVER

The experiments with the serum of typhus-fever patients will be discussed first.

Blood was taken from the patient by venepuncture, and after the separation of the clot, the serum was removed. From 3 to 5 c.c. were injected subcutaneously into virgin female guinea-pigs. The sera from the first 7 patients were injected fresh (without being inactivated). It was found, in agreement with the experience of Schloss, that fresh serum is toxic for guinea-pigs, causing either death or local ulceration or infiltration. The uteri of these animals failed to react on the addition of antigen to the surrounding Locke's fluid; this was due probably to the failure of absorption of the toxic fresh sera. This series of 7 cases was therefore discarded.

The sera of the 4 remaining cases were inactivated; with these sera, 7 experiments were made.

Two typical experiments will be described in detail. On October 29, Guinea-pig 23 received 4.5 c.c. of inactivated serum obtained from Case B59, 9 days after the crisis. On November 2, the guinea-pig was killed and the uterus suspended in the Dale apparatus. Figure 3 shows the reaction to 3 c.c. of antigen, indicating that the guinea-pig had absorbed the typhus antibodies from the injected serum. It is noteworthy that a second addition of 3 c.c. of antigen

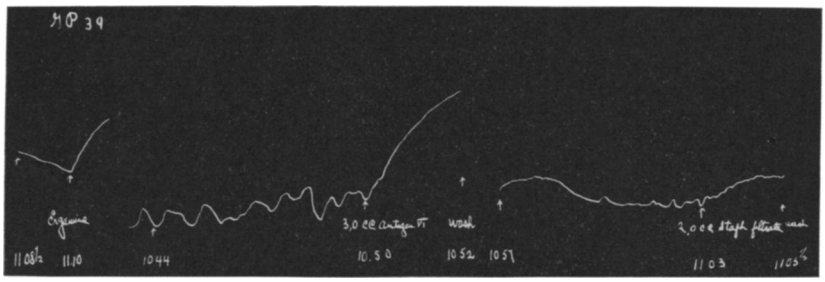

Fig. 3. Reaction to postcritical typhus serum. On October 29, Guinea-pig 23 received 4.5 c.c. of serum of Case B59, 9 days after crisis. November 2, uterus tested. Note reaction to 3 c.c. of antigen; also failure to react on the second addition of antigen, indicating desensitization. Good reaction to ergamine.

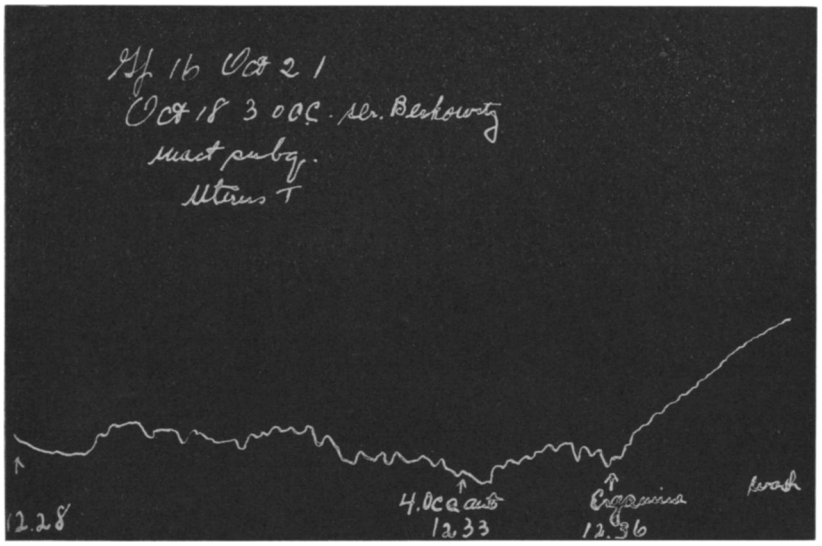

Fig. 4. Absence of reaction with typhus serum at height of disease. On October 18, Guinea-pig 16 received 3 c.c. of inactivated serum of Case B59. On October 21, uterus tested. Note absence of reaction to 4 c.c. of antigen.

failed to produce a contraction; the uterus had been desensitized by the first dose. The response to ergamine shows that the uterus retained its contractility.

Figure 4 illustrates the result of injecting serum from the same case at the height of the disease. On October 18, Guinea-pig 16 received 3 c.c. of inactivated serum obtained from Case B59 on the 10th day of illness at the height of the disease. There is no reaction to 4 c.c. of antigen, altho there is a sharp response on the addition of ergamine.

Of the 7 experiments in this series, 3 were made with sera obtained during the height or febrile stage of the disease (1st to 10th day). All were negative, indicating the absence of demonstrable antibody in the serum of typhus-fever patients during the height of the disease. In the remaining 4 experiments sera obtained from 4 to 9 days after the crisis were used. Three of these reactions were positive, indicating the presence of antibody in the postcritical period of typhus fever. The negative reaction occurred with serum sent to us from another hospital and in this case the possibility of a mistaken diagnosis exists. The significance of these findings will be discussed later.

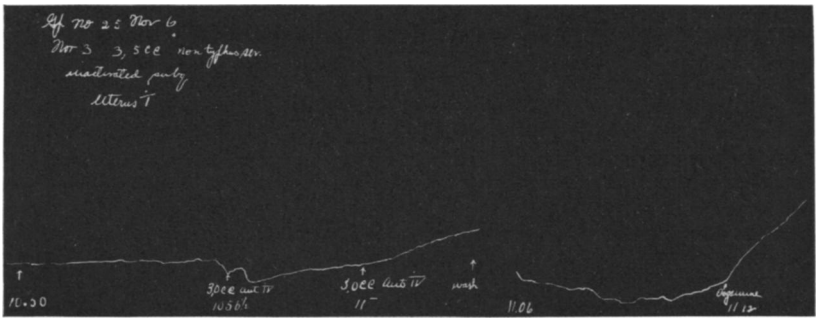

Fig. 5. Absence of reaction with nontyphus serum. On November 3, 3.5 c.c. of nontyphus serum injected subcutaneously. Note absence of reaction to 3 c.c. and almost negligible reaction to an additional 5 c.c.

As a control to these experiments, the blood of 6 individuals not suffering from typhus fever was tested (Figs. 2 and 5). Two of these were normal people, the remaining four were patients suffering from various febrile conditions, influenza, liver abscess, etc. The technic was identical with that used in the experiments with typhus-fever serum. There was a complete absence of uterine response in every case. Figure 5 illustrates a typical case. On November 3, Guinea-pig 25 received 3.5 c.c. of inactivated nontyphus serum subcutaneously. Three days later the animal was killed and the uterus was suspended in the Dale apparatus. There was no reaction to 3 c.c. or to 5 c.c. added shortly afterward. The reaction to ergamine indicated that the uterus retained its contractility.

CELLULAR ANTIBODIES IN GUINEA-PIGS

Ricketts and Wilder, in 1910, showed that the virus of typhus fever can be transmitted to guinea-pigs; that after an incubation period of from 7 to 10 days, the temperature rises, remains elevated for a period of from 7 to 14 days, and that recovery usually follows. Re-inoculation with typhus virus fails to cause a febrile reaction, thus indicating the development of immunity. In his studies on such immune guinea-pigs, Olitsky reached the following conclusion: "Altho the animal was proved immune, yet the serum contained no agglutinin or complement-fixing bodies. . . . It is most probable that the guinea-pig reacts to the typhus virus and develops subsequently a high grade of immunity by means of its tissue elements and only to a very slight degree by means of the circulating blood." If the immunity is indeed chiefly of a cellular nature, the Dale method is peculiarly fitted to the study of the immune process in the guinea-pig. The uterine-muscle preparation of a guinea-pig sick of typhus fever demonstrates by its contraction on the addition of typhus antigen, the existence of antibodies in the cells against Bacillus typhi-exanthematici.

The modus operandi of these experiments was as follows: Female virgin guinea-pigs were injected intraperitoneally with the virus of typhus fever. This virus was obtained originally from a case of endemic (New York) typhus fever in the wards of Mount Sinai Hospital in 1911. It has been kept alive in guinea-pigs since that time. The virus (which must have passed thru approximately 150 generations in guinea-pigs) has maintained its strength with remarkable uniformity; it regularly produced a temperature of about 6 days' duration after an incubation period of about 10 days when inoculated intraperitoneally into guinea-pigs.

Fourteen guinea-pigs, not including the controls, comprise this series. The animals were tested during the height or febrile stage of the disease and at varying intervals after the crisis. Two typical experiments will be outlined in detail.

On November 18, Guinea-pig 39 was injected intraperitoneally with typhus virus from Guinea-pig 353. After an interval of 10 days, it developed a temperature which remained elevated for 5 days. Five days after the crisis, the animal was killed and the uterus suspended in the Dale apparatus. Figure 6 shows a marked reaction on the addition of 3 c.c. of antigen. The contraction following ergamine indicates that the uterine muscle retained its contractility.

Figure 7 illustrates an experiment on a guinea-pig killed during the height of the disease. Guinea-pig 51 was injected intraperitoneally January 1 with 3 c.c. of typhus virus. January 13, the 3d day of fever, the animal was killed and the uterus was suspended in a Dale apparatus. On the addition of antigen there was a complete absence of response, and the contraction following ergamine indicates that the uterus retained its contractility.

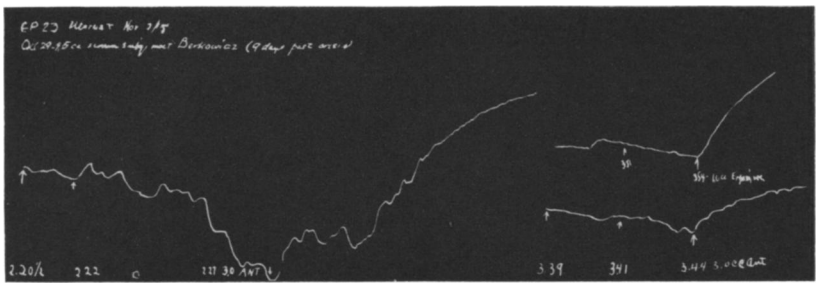

Fig. 6. Reaction in typhus-virus guinea-pigs after the crisis. Guinea-pig 39 showed typical febrile reaction to injection of typhus virus. Five days after the crisis, uterus tested. Note marked reaction to typhus antigen, negligible reaction to staphylococcus antigen, and good reaction to ergamine.

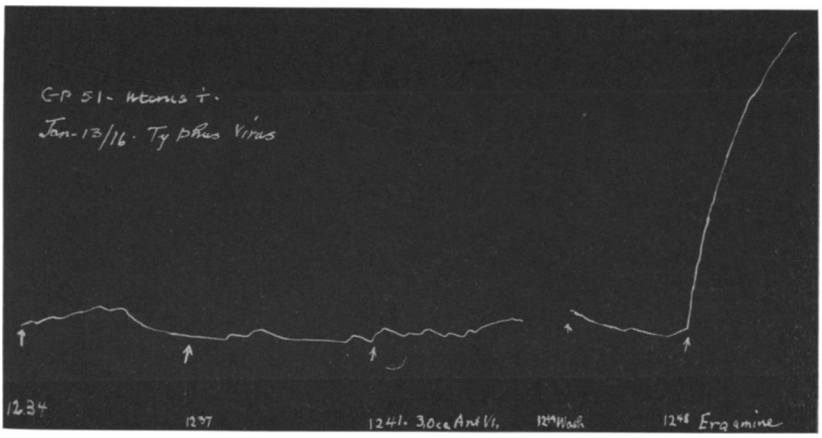

Fig. 7. Absence of reaction in typhus-virus guinea-pigs during the height of the disease. Guinea-pig 51 showed typical febrile reaction to typhus-fever virus. At the height of the fever, uterus tested. Note absence of response to antigen and good reaction to ergamine.

Of the 14 guinea-pigs in this series, 5 were tested during the height of the disease. Of these five none showed a reaction on the addition of antigen. Of the remaining 9, tested at varying intervals after the crisis, 7 showed a positive reaction. One of 2 guinea-pigs killed post-critically that failed to react, was killed only 2 days after the crisis. The seven guinea-pigs that showed a muscular response on the addition of antigen, were tested 3, 5, 8, 10, 12, and 18 days after the crisis.

To determine whether the reaction obtained in guinea-pigs convalescent from typhus fever was due to a specific response to the addition of typhus antigen, several antigens made from other organisms

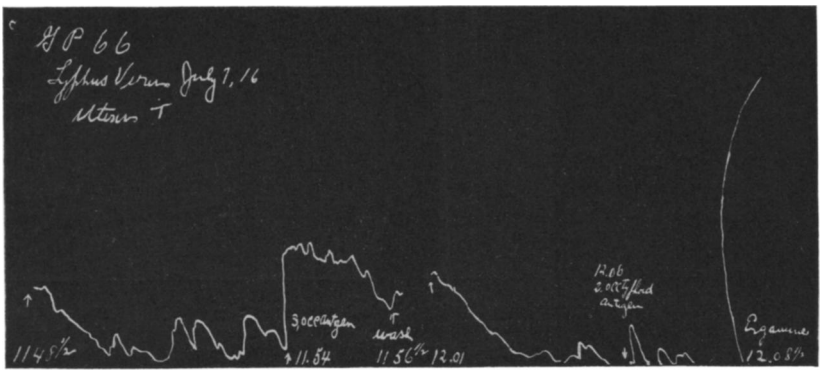

Fig. 8. Reaction to typhus antigen in typhus-virus animals after crisis, and failure to respond to typhoid antigen. Guinea-pig 66 reacted in typical manner to typhus virus. Uterus tested 8 days after the crisis. Note reaction to typhus antigen; also absence of response to typhoid antigen. Reaction to ergamine marked.

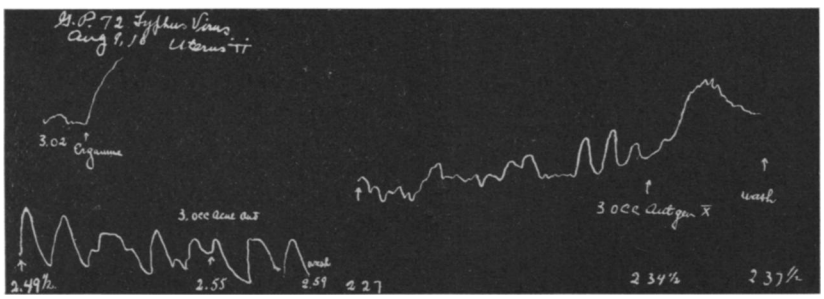

Fig. 9. Reaction to typhus antigen in typhus-virus animals after crisis and failure to respond to acne antigen. Guinea-pig 72 reacted in typical manner to injection of virus. Eight days after crisis uterus tested. Note reaction to 3 c.c. of typhus antigen and failure to respond to acne antigen. Reaction to ergamine good.

were used. Thus, antigens were made from M. aureus, B. typhosus, and B. acne by the method used in preparing typhus antigen. For the strains of B. acne we wish to express our indebtedness to Dr. E. P. Bernstein. The antigens were first tested against normal uteri to ascertain the correct dosage. Figures 6, 8, and 9 show the absence of response to the antigens mentioned, and repetition of the experiments confirmed these findings. The negative results with B. acne are particularly significant because of the slight similarity of the morphology of this organism to that of B. typhi-exanthematici. These results tend to corroborate the previous findings in regard to the specificity of the typhus bacillus in typhus fever.

DISCUSSION

The experiments thus detailed establish the fact that an anaphylactic antibody can be demonstrated in the serum of typhus-fever patients after the crisis and is not demonstrable during the height of the disease. This agrees with the finding of Olitsky that agglutinins, opsonins, and complement-fixing bodies are rarely present during the febrile stage of the disease, but are uniformly to be found after the crisis. From these facts one can not assume the entire absence of antibodies in the circulation during the febrile period. Weil has suggested, as a result of his work on unformed proteid substances and also on the blood of pneumonia patients, that other factors must be taken into consideration; that the presence of antigen (and in infectious diseases that means the specific microorganism or its derivatives) may prevent the demonstration of the antibody, which may nevertheless be present. These theories are particularly suggestive in relation to typhus fever, as Plotz has found the organism in the blood of patients 2 days after the crisis, when antibodies were already present in the serum.

It will be recalled that the previous workers have inferred that the immunity of guinea-pigs to typhus fever acquired in the initial attack must be ascribed to the development of cellular antibodies, because antibodies in the circulating blood could not be found. The present work demonstrates the presence in guinea-pigs of cellular antibodies against B. typhi-exanthematici. Here, also, the antibody could not be shown to exist during the febrile stage of the disease, but occurred only after the crisis.

CONCLUSIONS

Antibodies against Bacillus typhi-exanthematici, demonstrated by the Dale method, are found in the serum of typhus-fever patients after the crisis. These antibodies are not present in the serum during the height of the disease.

Antibodies against Bacillus typhi-exanthematici are found in the cells of typhus-fever guinea-pigs after the crisis. These antibodies are not demonstrable during the height of the disease.

The reaction in the serum of typhus-fever patients and in the cells of the guinea-pig is specific; that is, by the use of similar methods no antibodies to Bacillus typhi-exanthematici could be demonstrated in the

blood of normal individuals or of patients suffering from other infections. Guinea-pigs in the postcritical stage of typhus fever showed antibodies only to Bacillus typhi-exanthematici, and not to Micrococcus aureus, Bacillus typhosus, or Bacillus acne.

The results with the Dale method offer further evidence of the etiologic relationship between Bacillus typhi-exanthematici and typhus exanthematicus.